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Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	S. Ananth Karumanchi et al.	Confirmation No.:	6646
Serial No.:	10/624,809	Art Unit:	1647
Filed:	July 21, 2003	Examiner:	Ian D. Dang
Customer No.:	21559		
Title:	METHODS OF DIAGNOSING AND TREATING PRE-ECLAMPSIA OR ECLAMPSIA		

Mail Stop Amendment
Commissioner for Patents
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SECOND DECLARATION OF DR. S. ANANTH KARUMANCHI

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application.

2. I am a nephrologist in the Center for Vascular Biology at Beth Israel Deaconess Medical Center and an Associate Professor of Medicine, Obstetrics and Gynecology at Harvard Medical School. I am a co-founder of Nephromics, Inc., which holds a license to

the above-referenced application.

3. I have read the specification of the above-referenced patent application and the Office Action dated May 1, 2007.

4. The above-referenced application features claims directed to methods for the diagnosis of pre-eclampsia or eclampsia that include the detection of sFlt-1, free PlGF, or free VEGF polypeptides, wherein the free PlGF or free VEGF have the ability to bind to sFlt-1. The diagnostic methods can be performed using biological samples that include bodily fluids, cells, or tissue samples from the subject.

5. In the experiment described below, which was carried out in my laboratory and under my supervision, I show that sFlt-1 polypeptide can be detected in placental trophoblast cells, a cell derived from the placenta, using the techniques described in the specification and that the levels of sFlt-1 polypeptide are increased in placental trophoblast cells from pre-eclamptic subjects as compared to normotensive control subjects.

For this experiment, placental tissue was collected from pre-eclamptic and normal pregnant patients and used to isolate villous tissue pieces with an approximate weight of 10-20 mg. The villous tissue pieces were rinsed in phosphate-buffered saline to remove

contaminating blood and then cultured in DMEM media that was supplemented with 10% fetal bovine serum. Ten pieces of villous tissue from different segments of placenta were used for the estimation of sFlt1 production from one placental sample.

The villous tissue, containing placental trophoblasts, were grown under standard tissue culture conditions (5% CO_2) for 24 hours. Placental trophoblasts are the most common cell types found in the placenta. The conditioned media was collected after 24 hours and sFlt1 measured using ELISA. The level of sFlt1 (ng/ml) was normalized to the weight of the placental tissue (mg of tissue). The resulting data for six subjects in each group is presented graphically in Exhibit A, attached herewith, as mean ± S.D. (* represents $P<0.05$.)

6. As shown in Exhibit A, the levels of sFlt-1 detected in cells derived from the placenta of pre-eclamptic subject samples are increased as compared to the levels of sFlt-1 detected in cells derived from the placenta of normal reference samples.

7. At the time of filing, a skilled artisan would be able to apply these methods to compare the relative levels of sFlt-1, free VEGF, or free PlGF in a cell type or tissue type from a pre-eclamptic subject and a normal reference subject, as claimed.

8. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Oct 23, 2007
Date

S. Ananth Karumanchi, M.D.

Exhibit A